Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: A double-blind, placebo-controlled dose escalation study

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Abstract: BACKGROUND: Epicutaneous allergen administration using a patch may be an alternative to subcutaneous or sublingual immunotherapy. OBJECTIVE: To optimize treatment dose and to demonstrate the efficacy and safety of epicutaneous immunotherapy. METHODS: This monocentric, placebo-controlled, double-blind trial included 132 patients with grass pollen-induced rhinoconjunctivitis. In February 2008, patients were randomly allocated to receive placebo or 3 different doses of allergen. Before and during the pollen season 2008, patients received 6 weekly patches. Efficacy was assessed 4 to 5 months later (n = 110) and during the pollen season of the treatment-free follow-up year in 2009 (n = 93). The primary outcome was patient-reported changes in hay fever symptoms assessed by a visual analog scale. Secondary outcome measures were weekly visual analog scale symptom scores during pollen season, use of rescue medication, changes in conjunctival and skin reactivity, as well as safety. RESULTS: Hay fever symptoms during the pollen season were reduced by more than 30% in 2008 and by 24% in 2009 in the high-dose group as compared with that in the placebo group, and the alleviation of symptoms in the follow-up year was dependent on the treatment dose. Higher allergen doses were associated with drug-related adverse events (AEs), predominantly manifested by pruritus, erythema, wheal, or eczema. Eleven systemic AEs of grades 1 to 2 required treatment and led to study exclusion. The dropout rate due to AEs was 8.3%. No drug-related serious AE was recorded. CONCLUSION: Epicutaneous immunotherapy is safe and efficacious in a dose-dependent manner after 6 patches only.

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TITLE PAGE

Original Article

Epicutaneous Allergen-specific Immunotherapy Ameliorates Grass-Pollen Induced Rhinoconjunctivitis: Double Blind Placebo Controlled Dose Escalation Study

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ABSTRACT

Background: The only disease modifying treatment of allergies is allergen-specific immunotherapy, which today is administered either subcutaneously or sublingually. In a pilot study we have recently shown that epicutaneous immunotherapy using a skin patch may be a promising treatment alternative.

Objective: To find the optimal treatment dose and to demonstrate sustained clinical efficacy and safety of epicutaneous immunotherapy.

Methods: This monocentric, placebo-controlled, double-blind trial included 132 patients with grass-pollen induced rhinoconjunctivitis. In February 2008, patients were randomly allocated to one of four treatment groups (placebo, 10HEP, 50HEP, 100HEP, n=33 each). Before and during the pollen season 2008, patients received 6 patches in total. Treatment efficacy was assessed at the end of the pollen season 2008 (n= 110) and after a subsequent treatment-free follow-up year 2009 (n=93).

Results: A clear dose-dependency of patient self-reported hay-fever symptom alleviation was observed after the follow-up year, reaching statistical significance for the highest treatment-dose group (100HEP) as compared to the placebo group ($P<0.05$). Seasonal hay-fever symptom scores were reduced by more than 30% in 2008 and by 24% in 2009 in the 100HEP group as compared to the placebo group. Higher allergen doses were associated with more drug-related adverse events, predominantly presenting as local reactions, such as pruritus, erythema, wheal and eczema. Eleven systemic allergic reactions (grade 0 to 2) needed treatment and led to study exclusion (adverse event induced drop-out rate: 8.3%). No drug-related serious adverse events were reported.

Conclusion: Epicutaneous immunotherapy is safe and efficacious in a dose-dependent manner after 6 patches only.

Clinical implications

Epicutaneous allergen-specific immunotherapy is a convenient, safe and efficacious treatment for IgE-mediated allergies leading to symptom alleviation after a single-treatment season.

Capsule summary

Due to the poor acceptance of long-term allergen-specific immunotherapy, there is a need to improve treatment convenience by offering a treatment option that is both needle-free and also confers symptom amelioration after short treatment duration. We propose that epicutaneous-allergen-specific immunotherapy could be such an alternative.

Key words

Epicutaneous allergen-specific immunotherapy, epicutaneous immunotherapy, Patch immunization

66 **Abbreviations**

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68	AE	Adverse event
69	AUC	Area under the curve
70	BU	Biological unit
71	CI	Confidence interval
72	CPT	Conjunctival provocation test
73	EPIT	Epicutaneous allergen-specific immunotherapy
74	HEP	Histamine equivalent prick unit
75	PRO	Patient-reported outcome
76	SCIT	Subcutaneous allergen-specific immunotherapy
77	SD	Standard deviation
78	SIT	Allergen-specific immunotherapy
79	SLIT	Sublingual allergen-specific immunotherapy
80	SPT	Skin prick test
81	VAS	Visual analogue scale

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INTRODUCTION

Today, IgE-mediated allergies such as allergic rhinoconjunctivitis and asthma have reached a worldwide prevalence of 25%,[1-4] and are therefore called 'the new epidemics of the 21st century'.[5] Although, not life-threatening, allergic rhinoconjunctivitis significantly reduces quality of life leading to physical discomfort, emotional stress and impaired social activity.[6-10] Furthermore, allergic rhinoconjunctivits is associated with high socioeconomic costs, not only due to work absenteeism and decreased productivity but also since it represents a risk factor for disease progression to allergic asthma requiring long-term pharmacotherapy.[7] Despite insufficient symptom control with pharmacotherapy,[7, 11] only 5% of the patients suffering from allergic rhinoconjunctivitis decide to undergo allergen-specific immunotherapy (SIT),[12-13] which is the only disease-modifying and long-lasting treatment option.[14-15] Yet, the requirement for numerous doctor visits and the risk of treatment associated systemic allergic side effects limit the broad application of subcutaneous allergen specific immunotherapy (SCIT),[14, 16] which has been introduced a century ago[17] and still represents the 'goldstandard' route for SIT.[4, 18] Aiming to increase acceptance of SIT, tremendous effort has been put into the development of more patient-convenient and self-administrable treatment routes.[19-20] With the consented approval of sublingual allergen-specific immunotherapy (SLIT) as a valid needle-free treatment alternative to SCIT in 1998,[21] a new area of SIT has been initiated. Thanks to the excellent safety profile with rare systemic allergic side effects[12, 22] SLIT can be self-administered and therefore represents an increasing percentiage of SIT treatments in Europe.[18] However, up to 75% of patients report oral and gastrointestinal side effects[12] and treatment duration is not reduced but still requires daily intake of high allergen doses for three years, leading to considerable treatment costs.[12] Therefore, treatment compliance with SLIT is low and only 15% of the patients finish the the full course of treatment.[23]

We therefore propose epicutaneous allergen-specific immunotherapy (EPIT) as a novel needle-free and potentially self-administrable treatment route for SIT using topical application of an allergen-extract under a patch. Good accessibility, important immune-surveillance function[24] with high density of antigen-presenting Langerhans cells in the epidermis,[25] make this outermost layer of the skin a very attractive target organ for administration of SIT. Also, the lack of vascularisation of the epidermis[25] minimizes the risk for inadvertent intravascular allergen injection and therefore systemic allergic side effects. First evidence for the effectiveness of EPIT was provided in the 1950s by French allergologists, who successfully treated their patients by application of allergen drops on scarified skin.[26-27] Our group has recently revisited this approach and confirmed its effectiveness in a double blind placebo controlled pilot trial.[28] We replaced 'scarification' by a more gentle method to remove the stratum corneum, i.e. by adhesive tape-stripping. Physical removal of at least part of the stratum corneum is important not only to enhance antigen penetration [29] but also to activate keratinocytes to produce pro-inflammatory cytokines.[25] The present study aimed to define the optimal dose and safety of EPIT for grass pollen allergy in a larger patient population.

METHODS

Trial population

A total number of 157 subjects were screened and 132 patients aged between 18 and 65 years with a history of grass-pollen allergic rhinoconjunctivitis and positive reactions to grass-pollen extract in the skin prick test (SPT) and conjunctival provocation test (CPT) signed informed consent. Exclusion criteria were eczematous skin lesions on the upper arms; perennial allergic rhinitis; infectious rhinitis within the last two weeks, surgical intervention within the last 30 days; pregnancy or nursing; hypertension; history of HIV/AIDS; mastocytosis; malignancy; active infectious disease; significant cardiovascular, pulmonary, renal, hepatic, hematologic, autoimmune, neurological or psychiatric disease; moderate to severe asthma; intake of antihistamines with long half-live within the last 2 weeks or corticosteroids within the last 5 days; intake of contraindicated medication for specific immunotherapy such as β-blockers or angiotensin-converting enzyme/antgiotensin II receptor antagonists, tricyclic antidepressants; and participation in another clinical trial within the last 60 day. The study was reviewed and approved by the local ethics committee and notified by Swissmedic, the regulatory agency. ClinicalTrials.gov no. NCT00719511

Clinical trial design and study procedure

This single center phase I/II randomized, placebo-controlled, double-blind study was designed to test the efficacious dose range, tolerability and safety as well as the sustained treatment effect of EPIT. On the basis of a random-number table, 132 grass-pollen allergic patients were allocated to the placebo group (n=33), the low-dose grass-extract treatment group (10HEP, n=33), the medium-dose grass-extract treatment group (50HEP, n=33) or the high-dose grass-extract treatment group (100HEP, n=33).

The study was performed between February 2008 and November 2009 at the University Hospital of Zurich. The trial design is outlined in Table I. At the screening visit, patients having signed informed consent received a brief physical examination. Skin prick test (SPT) and conjunctival provocation test (CPT) were performed to confirm grass-pollen allergy and to determine the baseline values. At least four weeks before the start of the 2008 pollen season patients received the first patch. Prior to patch administration, the application site on the upper arm or shoulder was tape-stripped 6 times with a scotch tape (3M Company, St Paul, Minnesota, USA). Following such skin preparation, the allergen containing patch was applied and patients were observed in the trial facility for 30 minutes. All patients were handed an emergency-set containing corticosteroids (Prednisone [Streuli, Uznach, Switzerland]) and antihistamines (Semprex, [GlaxoSmithKline, Brentford, United Kingdom]). After 8 hours, the patch was removed by the patient. To assess local reactions, all patients were contacted by phone 48 hours after application of the first patch. With the start of the pollen season, the following patches were administered in weekly intervals (allowed range was 5 days to 2 weeks) until treatment completion after application of the 6^{th} patch. Each patch was administered at a different location on the upper extremity. If a local reaction occurred, patients were asked to take a photograph and to contact the study site. During the entire pollen season severity of hay fever symptoms was recorded weekly on a visual analogue scale and use of rescue medication was assessed daily. This study was conducted in accordance with the International Conference on Harmonisation guidelines on Good Clinical Practice and the Declaration of Helsinki.

Test drug, dosing and patch system

Grass allergen extract derived from pollen of Holcus lanatus, Dactylis glomerata, Lolium perenne, Phleum pratense, Poa pratensis and Festuca elatior dissolved in a glycerinated solution (MG51 Mezcal 6 Graminees, Inmunotek, Madrid, Spain) was used as the test drug.

Patches with different biological activity (10HEP = 1 x prick test concentration, 76 µg/ml; 50HEP = 1 x atopy patch test concentration, 378 µg/ml; 100HEP = 2 x atopy patch test concentration, 757 µg/ml) were produced by the Cantonal Pharmacy, Canton of Zurich (Zurich, Switzerland) through integration of the allergenic grass-extract into a patch system provided by Medanz Medical GmbH (Starnberg, Germany). The patch was 95 mm x 95 mm in size containing a centred 50 mm x 30 mm sized fibrous web with the adsorbed allergen extract.

Efficacy measurements

The main objective of this study was to evaluate the optimal treatment dose as well as treatment efficacy of EPIT after a one-year treatment period consisting of the application of 6 patches. The primary endpoint was the determination of general improvement / deterioration of hay fever symptoms after the treatment season 2008 and the treatment-free follow-up year 2009 when compared to previous years. Secondary outcome measures were average visual analogue scale (VAS) symptom scores obtained as weekly records during the pollen season, use of rescue medication and changes in CPT and SPT as well as safety measures.

Patient-reported treatment outcome: improvement / deterioration of hay fever symptoms on VAS

At visit 7 and visit 9 patients were asked to rate the general improvement / deterioration of hay fever symptoms on a 200 mm VAS ranging from -100 (worst conceivable symptom deterioration) over 0 (unchanged symptoms) to +100 (best conceivable symptom improvement, total symptom relief).

Symptom and medication diary

During the pollen seasons 2008 and 2009 patients were asked to record the severity of nasal itching, sneezing, nasal obstruction, rhinorrhea, itchy eyes, lacrimation, itchy ears, itchy palate and lung symptoms on a 100 mm VAS ranging from 0 (no symptoms) to 100 (most severe symptoms) on a weekly basis. To assess the average weekly symptom scores, the sum of all symptoms (except lung symptoms) was calculated and divided by the total number of symptoms. Concomitantly, the daily use of provided rescue medication was assessed. Antihistamines up to 1 tablet daily (Aerius, 5mg, [Essex, Luzern, Switzerland]), antihistamine eye drops up to 1 drop per eye twice a day (Opatanol, 1mg/ml, [Alcon, Hünenberg, Switzerland]), nasal corticosteroids up to 2 puffs per nostril twice a day (Nasonex, 50µg/dose [Essex]) and inhalative corticosteroids up to 2 puffs twice daily ([Symbicort, 200µg AstraZeneca, Zug, Switzerland]). Start and end of the grass pollen season (28 April – 29 July 2008 and 4 May – 5 July 2009, 9 weeks each) was determined according to the seasonal development of the pollen load as measured by the Swiss national weather service (MeteoSwiss).

Conjunctival provocation test

CPT was performed at baseline as well as at visit 7 and 9. Before testing, the patients were adapted to room temperature for at least 10 minutes. Unspecific hyperresponsiveness was excluded by administration of 50 µl of the diluent. Subsequently 50 µl of increasing allergenic activity of grass-pollen extract (MG 51 6 Graminees, Inmunothek, Spain, Madrid) was administered into the lower conjunctival sac every 10 minutes, while alternating between the two eyes. Challenge was stopped when a positive reaction defined as a total symptom score ≥3 was recorded. Conjunctival redness, tearing, itching, burning and swelling of the eyelids were assessed and graded each after the protocol of Abelson[30] (absent = 0, slight = 1, definite = 2, severe = 3). At screening, patients were challenged with 5, 50, 500, 5000 biological units (BU). At the post treatment visits, 12500 and 25000 BU were included as

additional challenge doses, if the lower doses (5 BU – 5000 BU) did not induce a positive reaction.

Skin prick test

SPT was performed at baseline as well as at visit 7 and visit 9. Subjects were skin-pricked with different concentrations of grass-pollen extract 0.05HEP, 0.5HEP, 5HEP, 50HEP (MG51 Mezcal 6 Graminees, Inmunotek, Madrid, Spain) on the volar forearm. At the screening visit, polysensitization was tested by performing SPT for mugwort, hazel, birch, alder, ash, cat hair, dog hair, dermatophagoides pteronyssinus, and dermatogphagoides farinae (Stallergènes, Antony, France). Histamine hydrochloride and normal saline (Stallergènes, Antony, France) were used as positive and negative control, respectively. The SPT results were assessed according to the guidelines of the American Academy of Allergy, Asthma and Immunology, considering an erythema of 3-5 mm and a wheal of 2-3 mm as a positive reaction[31]. To grade the reaction, the classification system of Ring[32] was adopted.

Safety assessment

Adverse events and serious adverse events were defined according to the International Conference of Harmonization of Good Clinical Practice guidelines. Systemic side effects related to EPIT were graded according to the guideline of the WAO[33]. Grade 1: Symptoms of 1 organ system (cutaneous, upper respiratory tract, conjunctival, other), grade 2: Symptoms of more than 1 organ system present or lower respiratory tract (< 40 PEF or FEV1 drop), gastrointestinal; Grade 3: lower respiratory tract (40% PEF or FEV1 drop), upper respiratory (laryngeal, tongue edema); Grade 4: respiratory or cardiovascular failure.

Statistical analysis

The sample size calculation was performed assuming a mean self-reported improvement of 29% (SD = 27%) of the placebo group and a mean self-reported improvement of 52% (SD = 27%) in at least one treatment arm. Thus, to yield a statistically significant result with a power of 82% (2-sided analysis with $\alpha = 0.025$) and an expected drop out rate of 10%, a sample size of 33 per arm (total: 132) was calculated.

The scores from the VAS for the improvement / deterioration of hay fever symptoms were analyzed using Mann-Whitney multiple testing with Hochberg procedure adjustment. For evaluation of the seasonal VAS symptom scores, the area under the curve (AUC), assessed out of the average weekly VAS scores, was calculated for each patient. Simultaneous upper one-sided 97.5% confidence limits for the ratios to placebo were calculated to identify the minimum effective dose to achieve a 30% improvement (ratio 0.7) of the average VAS symptom score compared to placebo. For clinically beneficial values, the upper one-sided limits should thus be smaller than 1. Daily medication use was compared between the groups using the Kruskal Wallis test. Equal distribution of local side effects at the patch application site with increasing patch number was tested by chi-sqare test. All subjects with at least one application of the patch were evaluated for efficacy in an intention-to-treat analysis. Similarly, all subjects receiving at least one patch were included in the safety analysis.

RESULTS

Patient characteristics

An overview over the participant flow during study progress is provided in Figure 1. From February to March 2008 a total of 132 grass-pollen allergic patients signed informed consent and were enrolled in the study. A total of 33 patients were randomly allocated to each of the four treatment arms. Mean treatment duration lasted 83 ± 3 days. In the placebo group, 30 patients received at least one patch during the pollen season 2008 and were therefore included in the intention-to-treat analysis. Similarly, 31 patients each of the 10HEP and 100HEP and 32 patients of the 50HEP group received treatment during 2008 and were analyzed for treatment efficacy (2008, n = 110 in total). For the follow-up, several patients were either unable to come to the follow-up visits in 2009 or they needed to be excluded from the study due to intake of not permitted medication. Thus, a total of 22 patients of each the placebo and the 10HEP treatment group as well as a total of 25 and 24 patients of the 50HEP and 100HEP group, respectively, were included in the efficacy analysis for evaluation of sustained treatment effect (2009, n = 93 in total). At baseline, the demographic- as well as the disease-specific characteristics were similar between the four treatment groups with regards to disease duration, allergic asthma and co-sensitization to other allergens. Disease severity, as well, revealed to be comparable at baseline between the groups, presenting all with a history of moderate to severe rhinitis according to the ARIA guidelines as assessed by VAS[34] (Table II).

Efficacy

Patient reported treatment outcome

As primary endpoint we assessed the general impact of EPIT on grass-pollen allergy by determination of the subjectively experienced improvement or deterioration of hay-fever

symptom severity as assessed by VAS. After the first year we observed a general improvement of hay fever symptoms in all the treatment groups, including the placebo group (Fig. 2). Yet, after the 'treatment-free' follow-up year, the marked placebo effect observed in 2008 vanished, revealing a clear dose-response relationship 2009 (median improvement placebo 30.73, 10HEP group 30.73, 50HEP group 53.13, 100HEP group 69.79) Symptom alleviation for the high-dose treatment group was statistically significant when compared to placebo treated group (placebo-10HEP: p=0.532; placebo-50HEP: p=0.141, placebo-100-HEP: p=0.017) (Fig. 2).

Weekly seasonal symptom and medication score

During the treatment season 2008 the average weekly rhinoconjunctivitis symptom score was reduced by 32% (estimate 0.68, upper 97.5% CI = 1.09) in the 100HEP treatment group compared to the placebo group (Fig. 3). After the treatment-free follow-up year, the 100HEP group still experienced a 24% reduction of the average seasonal symptom score (estimate 0.77, upper 97.5% CI = 1.60) as compared to the placebo group. Again, a dose response relationship was observed for the reduction of seasonal symptom scores in 2009. The use of rescue medication (Desloratadin [Aerius], Olopatadin [Opatanol], Mometasone furoate [Nasonex] and Budenoside with Formoterole [Symbicort]) did not significantly differ between the different treatment groups neither during the pollen season 2008 nor during the pollen season 2009.

Conjunctival provocation test and skin prick test

In the CPT, no statistically significant difference in the degree of improvement was observed between the different treatment groups. Likewise, no significant difference was seen in SPT reaction thresholds.

Safety

During the entire study period a total of 1566 AEs were reported, of which 825 (52.7%) were graded as likely/definite drug-related. The most frequent likely/definite drug-related AEs were pruritus (417 events, 26.6% of total AEs), erythma (90 events, 5.7% of total AEs), wheals (149 events, 9.5% of total AEs) or eczema (138 events, 8.8% of total AEs); all occurring at the patch application site. Pruritus, erythema, wheals and eczema were observed with increasing frequency the higher the allergen treatment dose (Fig. 4a). Interestingly however, the occurrence of such local drug-related AEs significantly decreased with each patch application in all the treatment groups ($P < 0.001$)(Fig. 4b).

Eleven patients (8.3%) stopped treatment due to a systemic allergic reaction, which necessitated patch removal and administration of corticosteroids and antihistamines. Of these 11 patients, 1 had received placebo, whereas 10 had been treated with allergen extract (Tab. IV). All reactions were rated as grade 1 or grade 2. All presented with cutaneous reactions such as sustained pruritus distant from the patch application site and sensation of heat, whereas four patients additionally suffered from rhinitis and cough originating in the upper airways, or vertigo. All these systemic reactions responded to treatment with corticosteroids and antihistamines. None of these reactions required administration of adrenaline and none of these reactions were life-threatening or needed hospitalisation at any time.

DISCUSSION

The fact that allergic diseases constitute a global health problem with considerable impact on socioeconomic life[4, 7] underlines the need to develop a novel and convenient route for SIT with high efficacy and broad acceptance. Since the burden of allergic diseases goes beyond symptom scores and medication use but also affects quality of life, we chose the Patient-Reported Outcome (PRO) measure: 'improvement / deterioration of hay fever symptoms by VAS' as primary endpoint. Masked by a high placebo effect during the treatment year, the dose-dependent increase of self-reported overall treatment success became obvious only after a treatment-free follow-up year. With a median improvement of 70%, statistical and clinical significance was reached for the highest treatment dose group (100HEP) in comparison to the placebo group. Interestingly, the assessment of the seasonal rhinoconjunctivitis symptom scores was less subjected to the placebo effect, as average symptom scores were reduced in the 100HEP by more than 30% (as compared to the placebo group), already during the treatment year and by 24% after the follow-up year.

Although statistical significance for self-reported symptom improvement was only reached for the high dose treatment group, it is important to consider that statistical significance does not parallel clinical treatment benefit under real-life conditions. Therefore, it has been proposed that every improvement of > 30% is clinically relevant. Correspondingly, an improvement between 30 – 45% is judged as 'little' treatment effect, whereas an improvement between 46 – 60% is assessed as 'moderate' treatment effect and an improvement > 60% as 'strong' effect.[35] According to such rating, treatment effect was strong in the 100HEP, moderate in the 50HEP group and absent in the 10HEP group. Strikingly, such clinically relevant effect was achieved through application of merely 6 patches during a single pollen season.

Overall, EPIT proved to be safe, but not without systemic allergic side effects, which required treatment and led to study exclusion in 9% of the treated population. Yet, all of these systemic allergic reactions were mild (grade I to grade II) and occurred within 45 minutes after patch application. Compared to conventional SCIT, which is associated with a 30% risk of systemic allergic reactions (graded from mild to life-threatening),[36] the safety profile of EPIT appears better. Local reactions at the patch application site were frequently reported and mostly presented as pruritus, erythema, wheal and eczema. Although bothersome, the appearance of an eczematous skin reaction demonstrates the elicitation of an allergen-specific T cell response.[37-38] Such cellular immune-activation and potential immune-deviation is a crucial mechanism in SIT, which aims at re-directing the allergic T-helper (Th) 2 response toward a Th1 or T-regulatory (Treg) cell response[39-40]. Interestingly, over time, we observed a decrease of such local treatment-associated AEs, which might indicate reduction of allergen-specific T cell reactivity and development of peripheral T cell tolerance. Since induction of T cell tolerance has been demonstrated to be a key mechanism of efficacious SCIT[39] and SLIT[41-43] it might also play an important role in EPIT. To investigate the immunological changes associated with EPIT, such as induction of blocking antibodies and peripheral T reg cells, a subsequent trial has been initiated (NCT00777374). First results are expected in 2011.

The present trial reproduces the results of our first and smaller clinical trial on EPIT[28] with a larger patient number. As an improvement over our first clinical trial we could reduce the number of patch applications from 12 to 6, and the duration of each single patch application from 48 hours to 8 hours.

In summary, our data provide evidence for the dose-dependent efficacy of EPIT as needle-free, rapid and self-administrable treatment option for IgE-mediated allergies. ‘Epicutaneous’ allergen-specific immunotherapy further extends the steadily growing application field of needle-free vaccination methods delivering the antigen via the skin. ‘Transcutaneous’

vaccination against infectious diseases such as travellers' diarrhea[44] and influenza[45] are examples of this novel and promising vaccination route. Furthermore, 'percutaneous' peptide immunization has been shown to be successful in cancer therapy.[46] In line with these, epicutaneous allergen-specific immunotherapy may considerably contribute to combat one of the 'new epidemics of the 21st century'. Its easy and painless administration might promote the increased prescription of SIT in children, which is an important goal, since SIT has the potential to stop the 'atopic march' and disease progression to asthma.[47] EPIT therefore has the potential to reduce the socio-economic burden of allergies when administered early in the course of the disease.[48]

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Table I. Study outline

Visit/time	Intervention/analysis
February/March 2008	Newspaper advertisement and telephone recruitment
Screening (March-April 2008)	Medical history and examination, CPT, SPT, Severity assessment of rhinoconjunctivitis with VAS
Visit 1 (March-April 2008)	First patch application Follow-up call after 48 h for record of local AEs
Visit 2 (April-May 2008)	Second patch application, AE records
Visit 3 – 6 (May-July 2008)	(Bi-)weekly patch application, AE records Weekly VAS symptom diary, Medication diary
Visit 7 (August-September 2008)	Medical examination, CPT, SPT, AE records, Recording of improvement / deterioration VAS
Visit 8 (March-May 209)	AE records
Pollen season 2009	Weekly VAS symptom diary, Medication diary
Visit 9 (September-November 2009)	Medical examination, CPT, SPT, AE records, Recording of improvement / deterioration on VAS

VAS = visual analogue scale, CPT = conjunctival provocation test, SPT = skin prick test, AE = adverse event

Table II. Baseline characteristics

	Treatment group			
Characteristics	**Placebo**	**10HEP**	**50HEP**	**100HEP**
Age (y), mean ± SD	39 ± 10.8	35 ±11.3	36 ± 10.2	36 ± 10.6
Female, n (%)	9 (27.3)	14 (42.4)	11 (33.3)	15 (45.5)
Duration of rhinitis (years), mean ± SD	23 ± 11.6	21 ± 11.2	21 ± 10.5	21 ± 10.6
Severity of rhinoconjunctivitis, mean total VAS ± SD	53 ± 14.9	47 ± 16.7	50 ± 17	51 ± 18.5
Severity of nasal symptoms mean VAS ± SD	60 ± 19.8	56 ± 19.5	57 ± 24.0	56 ± 18.9
Severity of ocular symptoms mean VAS ± SD	63 ± 20.1	52 ± 25.3	55 ± 19.0	52 ± 26.5
Severity oro-pharyngeal symptoms mean ± SD	28 ± 24.0	24 ± 24.2	31 ± 29.8	38 ± 26.7
Severity of lung symptoms mean VAS ± SD	38 ±33.7	24 ± 31.4	25 ± 28.9	24 ± 31.2
Seasonal asthma (%)	9 (27.3)	9 (27.3)	12 (36.4)	10 (30.3)
Monosensitized patients	7 (21.2)	5 (15.2)	11 (33.3)	7 (21.2)
Polysensitized patients	26 (78.8)	28 (84.8)	22 (66.7)	26 (78.8)
Baseline CPT threshold, mean ± SD				
5 BU	2 ± 6.1	1 ± 3.0	1 ± 3.0	1 ± 3.0
50 BU	5 ± 15.2	3 ± 9.1	8 ± 24.2	10 ± 30.3
500 BU	21 ± 63.6	21 ± 63.6	19 ± 57.6	11 ± 33.3
5000 BU	5 ± 15.2	8 ± 24.2	5 ± 15.2	11 ± 33.3
Baseline SPT, threshold, mean ± SD				
0.05 HEP	15 (45.5)	11 (33.3)	12 (36.4)	10 (30.3)
0.5 HEP	6 (18.2)	11 (33.3)	1133.3)	7 (21.2)
5 HEP	11 (33.3)	10 (30.3)	9 (27.3)	8 (24.2)
50 HEP	1 (3.0)	1 (3.0)	1 (3.0)	8 (24.2)

Table III. Adverse events

	Treatment groups			
Adverse events	**Placebo** (n = 33)	**10HEP** (n = 33)	**50HEP** (n=33)	**100HEP** (n=33)
Number of patients with AE, n (%)	33 (100)	33 (100)	33 (100)	33 (100)
Number of AE episodes	279	409	427	451
Causality assessment				
No relation, n (%)	14 (5.0)	14 (3.4)	18 (4.2)	11 (2.4)
Unlikely/Possible relation, n (%)	182 (65.2)	191 (46.7)	162 (37.9)	148 (32.8)
Likely/Definite, n (%)	82 (29.4)	204 (49.9)	247 (57.8)	292 (64.7)
Missing, n (%)	1 (0.4)	0	0	0
Severity assessment of Likely/definite drug related AEs				
mild, n (%)	60 (76.9)	135 (66.2)	139 (56.3)	147 (50.3)
moderate, n (%)	14 (17.9)	58 (28.4)	82 (33.2)	115 (39.4)
severe, n (%)	4 (5.1)	11 (5.4)	26 (10.5)	30 (10.3)

Table IV. Systemic allergic reactions necessitating intervention*

	Treatment groups			
Systemic allergic reaction	**Placebo** (n=33)	**10HEP** (n=33)	**50HEP** (n=33)	**100HEP** (n=33)
grade 1, n	1	1	3	2
grade 2, n	0	2		2
grade 3, n	0	0	0	0
grade 4, n	0	0	0	0

* treated with corticosteroids and antihistamines

Figure legends

Figure 1. Participant flow.

Flow diagram characterizing the study progress and participant flow through the different trial phases.

Figure 2. Patient reported treatment outcome.

Patients rated the general improvement / deterioration of hay fever symptoms on a scale from -100 (worst possible deterioration) to +100 (best possible improvement) during the seasons 2008 (treatment) and 2009 (follow-up). Box plots show the median, the 10^{th}, 25^{th}, 75^{th}, 90^{th} percentiles and outliers. Intergroup comparison by Mann-Whitney multiple testing with Hochberg procedure adjustment.*, $P < 0.05$

Figure 3. Mean VAS symptom score for pollen season 2008 and 2009

Mean weekly VAS symptom scores (except lung symptoms) of the 100HEP group compared to placebo (left axis). For each patient, the sum of all symptom scores was calculated and divided by the number of symptoms. The continuous line shows the weekly pollen counts in Zurich (right axis).

Figure 4. Local drug related adverse events

(A) Number of the most frequent local drug-related adverse events as reported in the different treatment groups. (B) Frequency of local side effects per patch sequence. Distribution of the frequency of local AEs was tested by chi-square test.

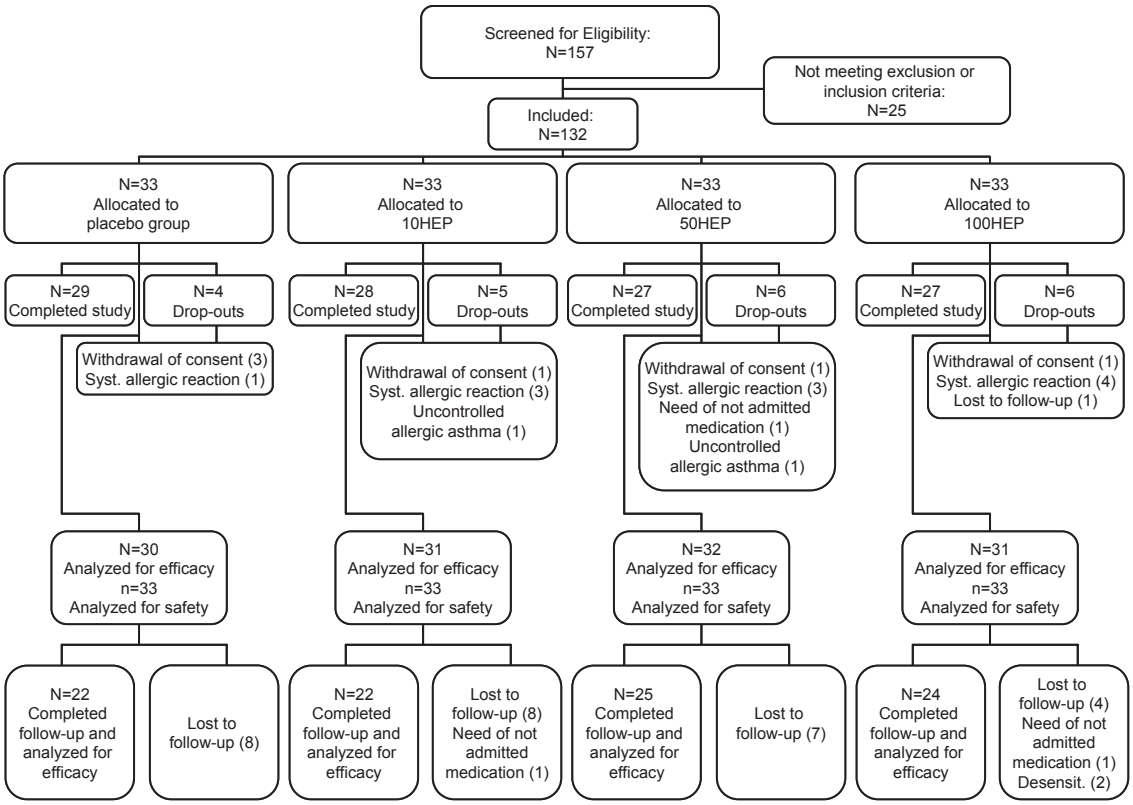
Screened for Eligibility:
N=157
Not meeting exclusion or
inclusion criteria:
N=25
Included:
N=132
N=33
Allocated to
placebo group
N=33
Allocated to
10HEP
N=33
Allocated to
50HEP
N=33
Allocated to
100HEP
N=29
Completed study
N=4
Drop-outs
N=28
Completed study
N=5
Drop-outs
N=27
Completed study
N=6
Drop-outs
N=27
Completed study
N=6
Drop-outs
Withdrawal of consent (3)
Syst. allergic reaction (1)
Withdrawal of consent (1)
Syst. allergic reaction (3)
Uncontrolled
allergic asthma (1)
Withdrawal of consent (1)
Syst. allergic reaction (3)
Need of not admitted
medication (1)
Uncontrolled
allergic asthma (1)
Withdrawal of consent (1)
Syst. allergic reaction (4)
Lost to follow-up (1)
N=30
Analyzed for efficacy
n=33
Analyzed for safety
N=31
Analyzed for efficacy
n=33
Analyzed for safety
N=32
Analyzed for efficacy
n=33
Analyzed for safety
N=31
Analyzed for efficacy
n=33
Analyzed for safety
N=22
Completed
follow-up and
analyzed for
efficacy
Lost to
follow-up (8)
N=22
Completed
follow-up and
analyzed for
efficacy
Lost to
follow-up (8)
Need of not
admitted
medication (1)
N=25
Completed
follow-up and
analyzed for
efficacy
Lost to
follow-up (7)
N=24
Completed
follow-up and
analyzed for
efficacy
Lost to
follow-up (4)
Need of not
admitted
medication (1)
Desensit. (2)

Figure 1

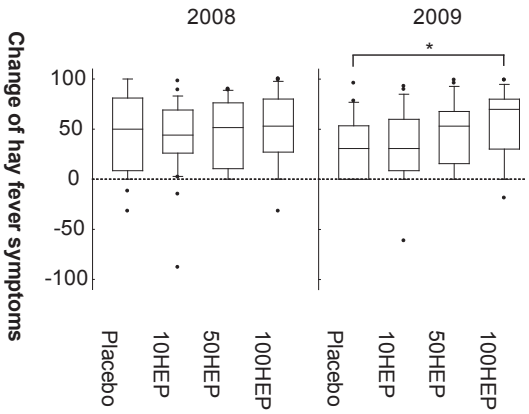
2008
2009
*
Change of hay fever symptoms
100
50
0
-50
-100
Placebo
10HEP
50HEP
100HEP
Placebo
10HEP
50HEP
100HEP

Figure 2

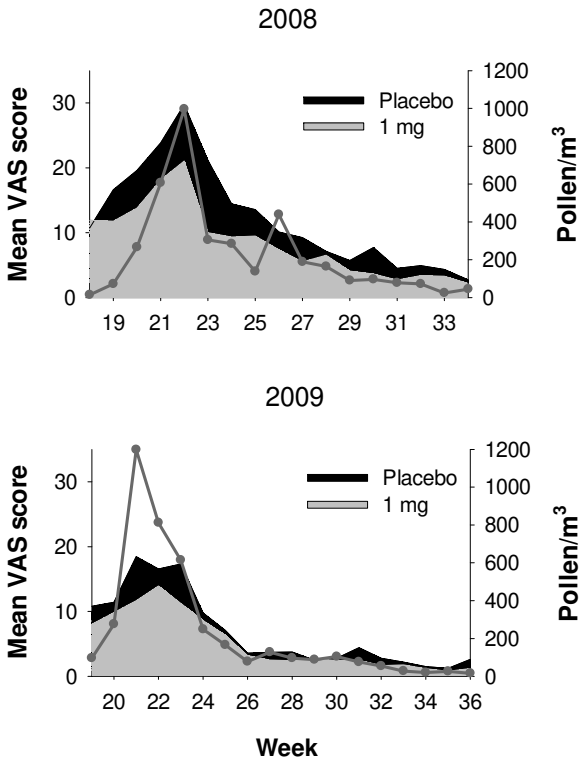
2008
Mean VAS score
Pollen/m3
Placebo
1 mg
0
10
20
30
0
200
400
600
800
1000
1200
19
21
23
25
27
29
31
33
2009
Mean VAS score
Pollen/m3
Placebo
1 mg
0
10
20
30
0
200
400
600
800
1000
1200
20
22
24
26
28
30
32
34
36
Week

Figure 3

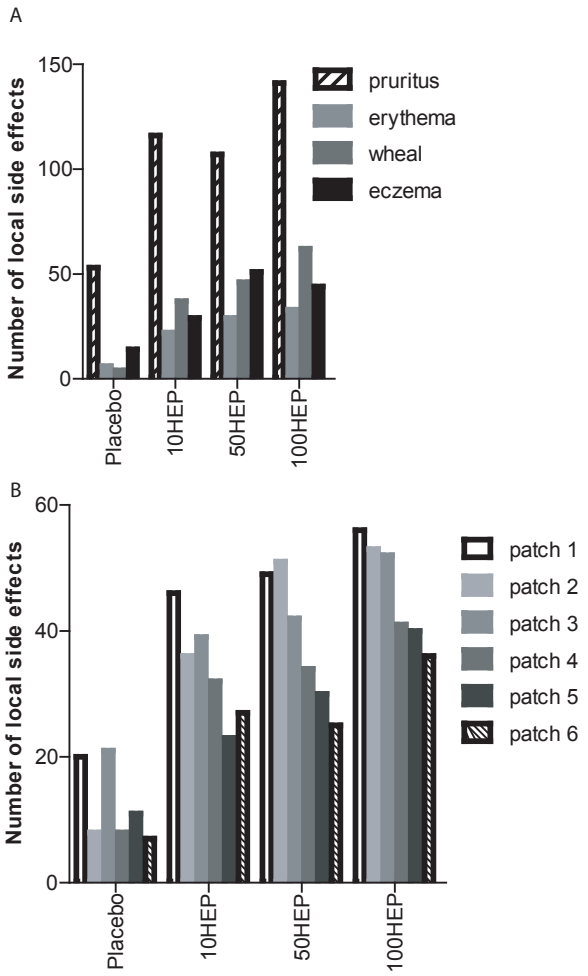
A
150
100
50
0
Number of local side effects
pruritus
erythema
wheal
eczema
Placebo
10HEP
50HEP
100HEP
B
60
40
20
0
Number of local side effects
patch 1
patch 2
patch 3
patch 4
patch 5
patch 6
Placebo
10HEP
50HEP
100HEP

Figure 4.